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The biocompatibility of titanium in a buffer solution: compared effects of a thin film of TiO_2 deposited by MOCVD and of collagen deposited from a gel

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Abstract This study aims at evaluating the biocompatibility of titanium surfaces modified according two different ways: (i) deposition of a bio-inert, thin film of rutile TiO_2 by chemical vapour deposition (MOCVD), and (ii) biochemical treatment with collagen gel, in order to obtain a bio-interactive coating. Behind the comparison is the idea that either the bio-inert or the bio-active coating has specific advantages when applied to implant treatment, such as the low price of the collagen treatment for instance. The stability in buffer solution was evaluated by open circuit potential (OCP) for medium time and cyclic voltametry. The OCP stabilized after $5 \cdot 10^4$ min for all the specimens except the collagen treated sample which presented a stable OCP from the first minutes. MOCVD treated samples stabilized to more electropositive values. Numeric results were statistically analysed to obtain the regression equations for long time predictable evolution. The corrosion parameters determined from cyclic curves revealed that the MOCVD treatment is an efficient way to improve corrosion resistance. Human dermal fibroblasts were selected for cell culture tests, taking into account that these cells are present in all bio-interfaces, being the main cellular type of connective tissue. The cells grew on either type of surface without phenotype modification. From the reduction of yellow, water-soluble 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT cytotoxicity test), MOCVD treated samples offer better viability than mechanically polished Ti and collagen treated samples as well. Cell spreading, as evaluated from microscope images processed by the program Sigma Scan, showed also enhancement upon surface modification. Depending on the experimental conditions, MOCVD deposited TiO_2 exhibits different nanostructures that may influence biological behaviour. The results demonstrate the capacity of integration in simulated physiologic liquids for an implant pretreated by either method.

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Introduction

Changes in surface chemistry or topography influence biocompatibility. The success of many metallic biomaterials is in direct relation with surface treatments applied in order to improve cell adhesion and proliferation in in vitro experiments [1]. Biocompatibility should be evaluated by testing citotoxicity of materials and corrosion resistance in a buffer solution.

Titanium is a widely used biomaterial. Various surface treatments like mechanical treatment, electrochemical treatment (anodizing), heating, laser treatment, ultrapassivation, nitriding, ion implantation, ceramic coating through thermal oxidation (rutile mainly), plasma coatings, calcium phosphate coatings have been all studied to achieve desired surface properties [2–7]. Since interactions between cells and titanium implants occur at their interfaces, surface

characteristics of titanium are essential. Despite the excellent biocompatibility of thin native oxide film on titanium implants, native titanium oxide is known to seldom bond chemically to bone tissue and is often presented as an inert ceramic biomaterial [8]. Several in vitro and in vivo studies showed the effect of coating composition on Ti surface: deposited rutile and anatase compared to natural TiO_2 show enhanced bone-like precipitation at the surface in simulated body fluids [6, 9]. The MOCVD technique has been recently applied to the growth of titanium dioxide coatings [10], which successfully passed several biological tests both in vitro [11, 12] and in vivo [13].

Recently, researchers tried to coat titanium with collagen to investigate its beneficial effect on biocompatibility [14–16]. Collagen is a biocompatible and bioactive polymer, either alone or in combination with other materials [15]. It possesses a prominent position in the field of tissue engineering. Tissue engineering being a development of new materials or devices capable of specific interactions with biological tissue, these materials may be based entirely on naturally occurring molecules [17].

In this paper we present preliminary results of a comparative investigation of the electrochemical behaviour and of the biocompatibility of titanium surfaces modified by deposition of either a collagen film or a TiO_2 thin film grown by the technique of MOCVD. The numerous MOCVD experimental parameters (precursor formula, deposition temperature, total pressure, flow rate(s), molar fraction of the precursor, and possible use of a reactive atmosphere) can be modified to obtain diversely nanostructured deposits with varied anatase/rutile ratios. This will be illustrated in this paper. Therefore MOCVD is a choice technique to study the influence of varied nanostructures and/or of the allotropic composition on biological behaviour. Moreover, when applied at low pressure, the MOCVD technique is well adapted to the conformal coating of objects with complex surfaces as are prostheses and implants.

The relevance of the present work is to give new facts from surface materials science (including surface treatment and surface analysis) and from biocompatibility testing to improve the behaviour of implant materials with either a bio-inert coating (TiO_2) or a bio-interactive coating (collagen).

Experimental

Preparation and surface treatment of titanium discs

Titanium rods were acquired from Institute for Non-ferrous and Rare Metals, Bucharest (Romania). Composition: 0.056% N_2; 0.015% Fe; 0.205% O_2; 0.015% H_2; 0.09% Al; 99.619% Ti. The working electrodes prepared from these rods consisted of flat discs 1 mm in height and 12 mm in diameter.

The surface of the discs were mechanically polished with emery paper with different grain sizes, washed with distilled water, etched in 3% HF / 20% HNO_3 for 10 min, degreased in benzene for 5 min, prolongedly rinsed with distilled water and dried in hot air.

Two types of surface modification were performed:

- *Biochemical surface modification* (BSM) by depositing a collagen thin film from a collagen gel containing 2.08 g of dry substance per 100 g of gel [18]. Two successive thin layers were deposited on previously mechanically treated Ti surfaces. The thickness of the coatings was 50 μm, as determined with the scale of an optic microscope. The samples will be referred to as Ti-col.
- *Chemical vapour deposition from a metal-organic precursor* (MOCVD) of a thin film of TiO_2. The experimental assembly has been described elsewhere [19]. The precursor was titanium isopropoxide, $Ti(O^iPr)_4$ (TTIP). The deposition parameters used in this study are presented in Table 1. Film thickness was estimated from the weight of the deposit, by weighing the substrate before and after coating.

Table 1 Deposition parameters (total pressure, deposition temperature and deposition time) and some film features for the MOCVD treated samples

Sample	P (torr)	T (°C)	Time (min)	Thickness (μm)	Composition	Roughness (nm)
Ti-7	20	400	180	1.40	A(t)–r rutile traces	R_a = 30
Ti-10	20	500	180	6.40	R(t)–A(t) rutile predominant	–
Ti-12	20	600	180	0.80	R(t)–A(t) rutile predominant	–
Ti-30	760	400	60	~2	A(59%)–R(41%) no texture	–
Ti-34	760	600	45	~1	A(56%)–R(44%) no texture	R_a = 200

A, R (a, r) stand for large (tiny) amounts of anatase and rutile respectively in the film. Film texture (t) precluded compositional analysis from XRD patterns for samples prepared at low-pressure. For the samples grown under atmospheric pressure, composition were estimated from the intensities of reflections 110 for rutile and 101 for anatase, using the formula: anatase weight % = 100 / [1 + 1.333 I(110)/I(101)], see [20].

Electrochemical procedures

The electrochemical experiments were all performed at room temperature (25 °C), in a naturally aerated buffer solution (NaCl, 8.74 g/L; $NaHCO_3$, 0.35 g/L; $Na_2HPO_4 \cdot 12H_2O$, 0.06 g/L; NaH_2PO_4, 0.06 g/L; pH = 6.5). This buffer solution contained components of simulated bio-liquid such as Ringer's, Hank's, and artificial saliva. For each sample the following sequence of operations was run:

- *open circuit potential* (OCP) measurements using MATRIX model 20 with an Ag/AgCl electrode as reference
- *potentiodynamic polarization experiments* in the buffer solution for untreated and treated metallic substrates, using a PAR model 179 potentiostat with a computer interface. These experiments were realized in a three-electrode cell containing 250 mL of electrolyte. Platinum and Ag/AgCl electrodes served as an auxiliary electrode and a reference electrode respectively. Cyclic curves in the buffer solution were registered between –800 mV and +4000 mV, at a scan rate of 2 mV/s. Prior to measurements, the specimens were maintained in the solution for 30 min. In order to obtain reliable results, the potentiodynamic polarization curves were recorded three times. Corrosion potential (E_{corr}), corrosion current density (I_{corr}) and polarization resistance (R_p) were derived from these curves using the linear polarization method.

X-ray diffraction and scanning electron microscopy

Grazing incidence ($\Omega = 2°$) X-ray diffraction (XRD) was performed with a Seifert 3000 TT diffractometer, using monochromatized CuKα radiation. Surfaces of the MOCVD samples were observed by scanning electron microscopy (SEM) using a LEO 435 VP apparatus equipped with an X-ray dispersive spectroscopy (EDS) analyzer.

Fibroblasts growth and proliferation

Human dermal fibroblasts were isolated using the explant technique. Some pieces of skin were placed in 35 mm Petri dishes and maintained alive in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 15% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 mg/ mL streptomycin. Explants were incubated at 37 °C in a humidified atmosphere with 5%CO_2. When the culture surface was filled by fibroblasts, the explants were removed and the primary culture was incubated for 24 h. After 2–3 days the medium was renewed by additional DMEM + 10% fetal bovine serum + antibiotics. After about 7 days, the fibroblasts became confluent and then the passages were made. Cells were used between the fifth and ninth passage and were grown as monolayer in Petri dishes.

The test discs (12 mm in diameter and 1 mm in height) were degreased with 70% ethanol, washed with an ultrasonic bath and sterilized by UV irradiation. In order to minimize the possible effects of TiO_2 photocatalysis [21], the test discs were washed with DMEM medium for 3 days. The human dermal fibroblast cells were seeded on the film at a density of 1×10^5 cells/ mL and cultured in order to check cell viability.

The methodology for in vitro experiments indicates the measurement of cytotoxicity as a convenient biological evaluation using direct contact methods with human dermal fibroblasts. The experimental cell culture environment was DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 mg/ mL streptomycin. The cells culture medium was renewed once every 2–3 days and incubated at 37 °C (the human body normal temperature) in a humidified atmosphere with 5% CO_2.

Morphological evaluation and viability tests

Morphological evaluation consisted in direct observation with an inverted microscope Nikon (Eclipse TS-100-F) equipped with an automated photo camera and a digital camera (Nikon Cool Pix 4500).

The tetrazolium-based colorimetric assay is a quantitative method to estimate cell proliferation, cell viability and cell citotoxicity [22]. This method is based on the reduction of yellow, water-soluble 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into dark-blue, water-insoluble formazan. The reduction is produced by a mithocondrial enzyme (succinat dehidrogenase) and is a parameter for cellular mithocondrial integrity. The amount of formazan generated is directly proportional to the number of viable cells. The insoluble formazan dissolves on addition of isopropanol, dimethylsulfoxyde or some other organic solvents. The optical density is evaluated by spectrophotometry, resulting in a relationship between absorbance, colorant concentration and number of active cells. The measurement is made on suspended cells, seeded on a multi-well plate (1,000–100,000 cells/well) and then incubated at 37 °C, in humidified atmosphere with 5%CO_2. After different periods of time (6–24 h), 200 µL /well of MTT solution (0.5 mg/ mL) were added to the culture medium and incubated again for 3 h. The medium with MTT was removed, and 200 µL/well of isopropanol were added in order to dissolve crystallized formazan. Then the absorbance was measured in a spectrophotometer at a wavelength of 570 nm.

Image analysis

Image analysis, very often used in cell research, [23] facilitated qualitative and quantitative characterization of cells culture. Image analysis was performed with the Sigma Scan program to estimate diameters and areas of the cells and cell spreading. Numerical analysis includes primary statistical analysis, sorting and data classification in order to remote irrelevant results, data transformation and identification of significant relations, the use of graphic representation or exported data in statistical program. In this study, the statistical treatment was done with the MedCalc program.

Results

Morphological analysis of TiO_2 deposited by MOCVD

MOCVD grown titania thin films differ in morphology and in allotropic composition depending on the growth conditions. Figure 1 shows SEM micrographs with the same magnification for samples Ti-7, Ti-10, Ti-12 and Ti-30.

The two films prepared at atmospheric pressure (Ti-30 at 400 °C and Ti-34 at 600 °C) are mixtures of anatase (~60 wt. %) and rutile (~40 wt. %) from X-ray diffraction (Table 1). They show no marked preferential orientation. The film grown at 600 °C is slightly rutile richer than at 400 °C. Despite different thicknesses (~2 μm for Ti-30, ~1 μm for Ti-34), both films have similar morphologies consisting in dendritic crystals (Fig. 1d).

The films prepared under reduced pressure show different morphologies (Fig. 1a–c) and different allotropic compositions. These differences may result from the different temperatures of preparation and/or from the different times of deposition hence different thicknesses. The films show preferential orientations so that it was not possible to determine the allotropic compositions from intensity measurements (Table 1). Ti-7 grown at 400 °C is 1.4 μm thick. It consists of anatase with X-ray diffraction detectible trace amounts of rutile. The SEM micrograph (Fig. 1a) shows small crystals, 100–300 nm in size, rather uniformly and densely spread with isolated protuberant bigger crystals. The film is strongly oriented from X-ray diffraction, but the grazing incidence technique does not allow to precise the preferred orientations. Contrastingly enough, Ti-12 grown at 600 °C and 0.8 μm thick presents a smooth surface (Fig. 1c). From X-ray diffraction, it consists of textured rutile and anatase, rutile being widely in excess. Ti-10 grown at 500 °C is 6.4 μm thick. It is a mixture of rutile as the predominant species and anatase, both species being strongly textured. The SEM micrograph (Fig. 1b) shows well-separated stacks of crystals about 1 μm wide.

Electrochemical evaluation

Metallic biomaterials used in orthopedic and dental surgery should be in a passive state [24]. The evolution of the open circuit potential vs. time was followed during about three months for samples Ti, Ti-col, Ti-10, Ti-12 and Ti-30 (Fig. 2). The stability of titanium in various simulated bio-

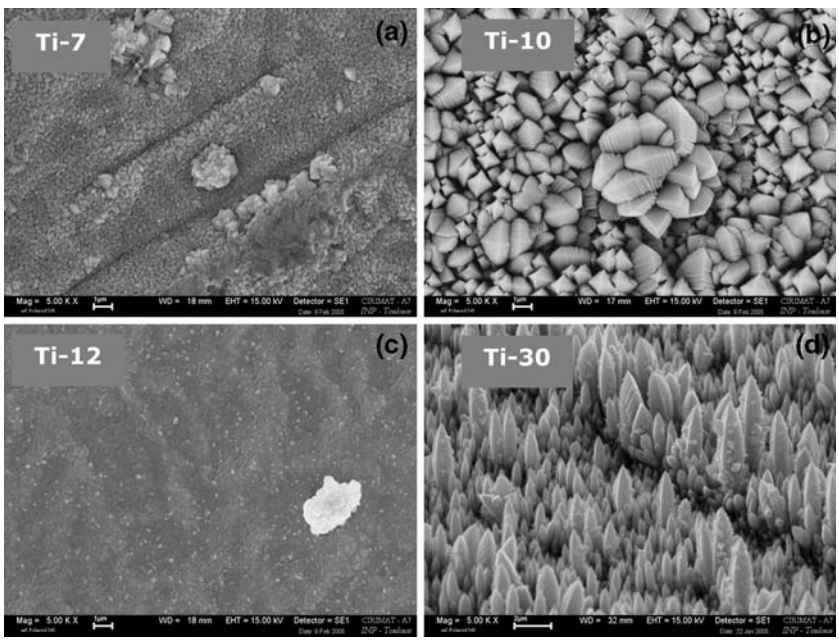

Fig. 1 SEM micrographs of MOCVD grown TiO_2 thin films: (**a**) Ti-7 (scale 1μm), (**b**) Ti-10 (scale 1μm), (**c**) Ti-12 (scale 1μm), (**d**) Ti-30 (scale 1μm)

liquids is due to a protective mixture of titanium oxides, mostly TiO_2. All studied samples possess a protective oxide film. This is evident for the MOCVD treated samples. For the titanium samples, a very thin layer of TiO_2 spontaneously forms on exposition to air before immersion in the buffer solution. Ti-col samples are covered with a film of collagen 50 μm thick. The electrode potential increases and reaches a steady state when the protective film (TiO_2 or TiO_2 + collagen) is in equilibrium with the simulated physiological medium after some sequences of dissolution and regeneration of TiO_2, as will be discussed thereafter.

The regression equations to predict long time evolution (Table 2) were derived from the statistical analysis of numeric results.

Cyclic polarization curves were recorded for Ti, Ti-col, Ti-10 and Ti-30 (Fig. 3) to determine the electrochemical specific parameters I_p (penetration index), R_p, I_{cor} in physiological media for Ti and treated Ti samples. These curves do not present active–passive transition regions. They are passive from the beginning because the oxide layer is cathodic polarization resistant. The electrochemical stabilities of the films are evaluated by the parameters listed in Table 3.

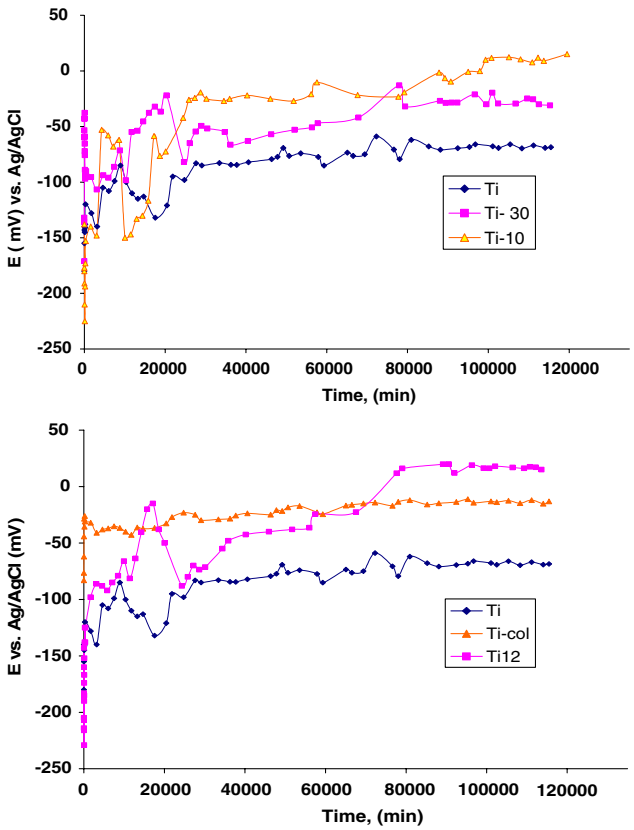

Fig. 2 Comparative evolution of OCP vs. time for samples Ti, Ti-10, Ti-12, Ti-30 and Ti-col

From Table 3, all samples show very low passive current density indicating they are highly corrosion resistant. Significantly higher R_p and lower I_{cor} values for the coated surfaces with respect to the uncoated one indicate that the coatings do improve corrosion resistance. The MOCVD samples possess the highest corrosion resistance. The structure of the MOCVD grown films appears more stable than that obtained by oxidation in air. The predominant structure is anatase in sample Ti-30 and rutile in sample Ti-10. From Table 3, of these two passive films, the anatase one is the most corrosion resistant in the studied environment.

Fibroblasts proliferation

After 3 days of culture on either treated and non treated surfaces, the fibroblasts were analyzed by observing their morphologies with an inverted microscope (Fig. 4). Fibroblast morphology did not vary significantly during the culture period. The cells showed normal behaviour and no change in phenotype. They were not always uniformly distributed on the plate.

Cell proliferation and cell viability were measured by the MTT method. As an example, Table 4 shows a comparison of viability data for cells grown on titanium, on titanium coated with MOCVD TiO_2 (Ti-7 & Ti-30), on titanium covered with the collagen gel and on the reference sample. The fibroblasts show better viability on MOCVD-modified surfaces than on mechanically treated titanium. Sample Ti-34 leads to the highest viability value, 78.9.

Cell diameters and areas were deduced from image analysis. Their statistical analysis (Fig. 5) lead to cell spreading that is the ratio between the total surface of the cells and the host area surface. Cell spreading values are gathered in Table 4. From the measured area of adhering fibroblasts, the largest adhesion areas were found for the cells cultured on MOCVD-TiO_2 and collagen treated surfaces (Table 4). This means that the cells are adherent on the surface and present a trend to spread all over the plate surface. High area values represent groups of cells in close contact to each other.

Discussion

In this study two kinds of titanium surface preparation were used in order to possibly get a better biocompatibility response: MOCVD coating with a thin film of TiO_2, and the covering with a thin film of collagen. The excellent corrosion resistance of Ti is well known to result from the existence of a few nanometers thick native oxide film. This essentially amorphous film proves a certain degree of short-range order in the nanometer range [25]. The com-

Table 2 Regression equations and determinant coefficients for the samples

Sample	Regression equation	Determinant coefficient
Ti	Y = –189.6708 + 23.3528 Log(X)	0.87
Ti-30	Y = –105.6502 + 12.9722 Log(X)	0.53
Ti-12	Y = –263.7190 + 51.3978 Log(X)	0.85
Ti-10	Y = –280.5259 + 52.9962 Log(X)	0.74
Ti-col	Y = –69.0004 + 10.2106 Log(X)	0.65

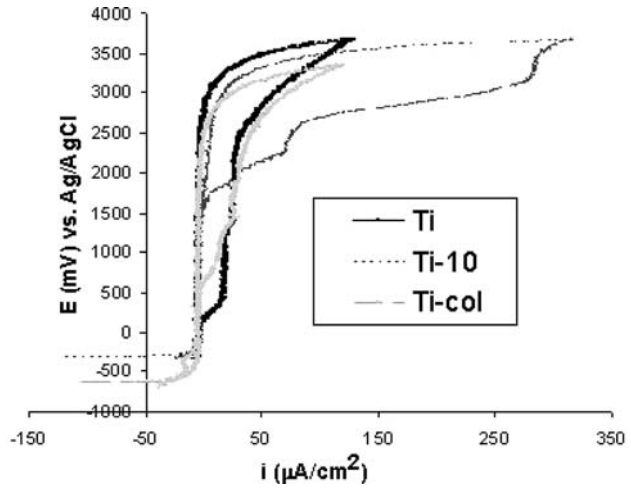

Fig. 3 Cyclic polarization curves for samples Ti, Ti-10 and Ti-col

position of the passive layer is widely documented in the literature [26–28]. This oxide also shows good tissue biocompatibility [25]. The effect of the different crystallographic forms of titanium dioxide on cells response was investigated by Zhao L. et al. for rutile powder, and for anatase and rutile ceramic discs [29]. As a recently used technique to grow TiO_2 for biocompatibility [13], MOCVD has the advantage of producing thin films with variable and reproducible anatase/rutile ratios, surface morphologies, and thicknesses. Most interestingly, MOCVD makes it possible to uniformly coat the complex surfaces of implants. In the present study, different mixtures of rutile and anatase were obtained by varying CVD experimental parameters. Cell viability has been measured for a sample consisting of anatase and traces amount of rutile (Ti-7) and for a sample consisting of 56% anatase / 44% rutile (Ti-34). Measurements on a film of rutile are still to be made. It can be expected that anatase is more favourable to cell viability than rutile because it generally gives less dense hence more porous films. Figure 1b, c shows that the samples in which rutile predominates can have a very porous surface (Ti-10) or a very smooth one (Ti-12). Viability tests on rutile should show if porosity is the main factor or if the crystallographic form also plays a role.

For all the samples, the open circuit potential sharply increased during the early hours, suggesting that a passive film rapidly formed on the metal surface. Then the OCP tended to stabilization, with however significant differences from one sample to the other. For the collagen treated sample, the OCP rapidly reached a rather stable value, after very smooth oscillations during the first 10 h or so. For the other samples, the OCP showed an overall increasing regime, with a few sharp oscillations over the first 2 weeks. Stable values were not reached before at least 2 months or so: 54 days for Ti-12 and Ti-30, 59 days for Ti, 69 days for Ti-10. This later behaviour has already been reported for titanium in several simulated physiological media, with just some differences concerning the time to reach the equilibrium [24]. MOCVD samples with rutile as the predominant form (Ti-10 and Ti-12) stabilized to more electropositive values than mechanically polished titanium, MOCVD treated sample with predominant anatase (Ti-30) and collagen treated titanium.

The cyclic curves do not show film breakdown. The current density presents low values for all the samples. The electrochemical parameters are better for both types of modified surfaces, indicating that the two types of treatment decrease the corrosion rate. The corrosion rate is very small for every sample, indicating very good film stability in the following order: Ti < Ti-col < Ti-10 < Ti-30 (1).

As discussed in papers on the characterization of titanium oxide by electrochemical impedance spectroscopy, the film formed on titanium under different exposure conditions is essentially titanium dioxide TiO_2 and comprises a dense inner layer and a porous outer layer [30, 31]. On both untreated and treated Ti samples, the fibroblasts are scattered and exhibit a polygonal elongated shape, with no significant morphological changes (Fig. 4). Cell growth data correlate with the electrochemical data, and reveal a better viability for treated Ti samples. UV irradiation (exposure to day light, sterilization procedure) is known to make TiO_2 surfaces highly hydrophilic. This may partly account for the increased cell viability [6, 32] on the MOCVD treated samples.

Table 3 Electrochemical parameters from cyclic curves

Sample	E_{cor} (mV)	I_{pas} (µA/cm^2)	I_{cor} (A)	R_p (Ω)	I_p (mm/year)
Ti	–131	26	$4.81 \cdot 10^{-6}$	5405	$4.17 \cdot 10^{-8}$
Ti-col	617	25	$3.17 \cdot 10^{-6}$	8196	$2.75 \cdot 10^{-8}$
Ti-10	1570	0.63	$2.02 \cdot 10^{-6}$	12820	$1.75 \cdot 10^{-8}$
Ti-30	1172	11	$1.48 \cdot 10^{-6}$	17543	$1.28 \cdot 10^{-8}$

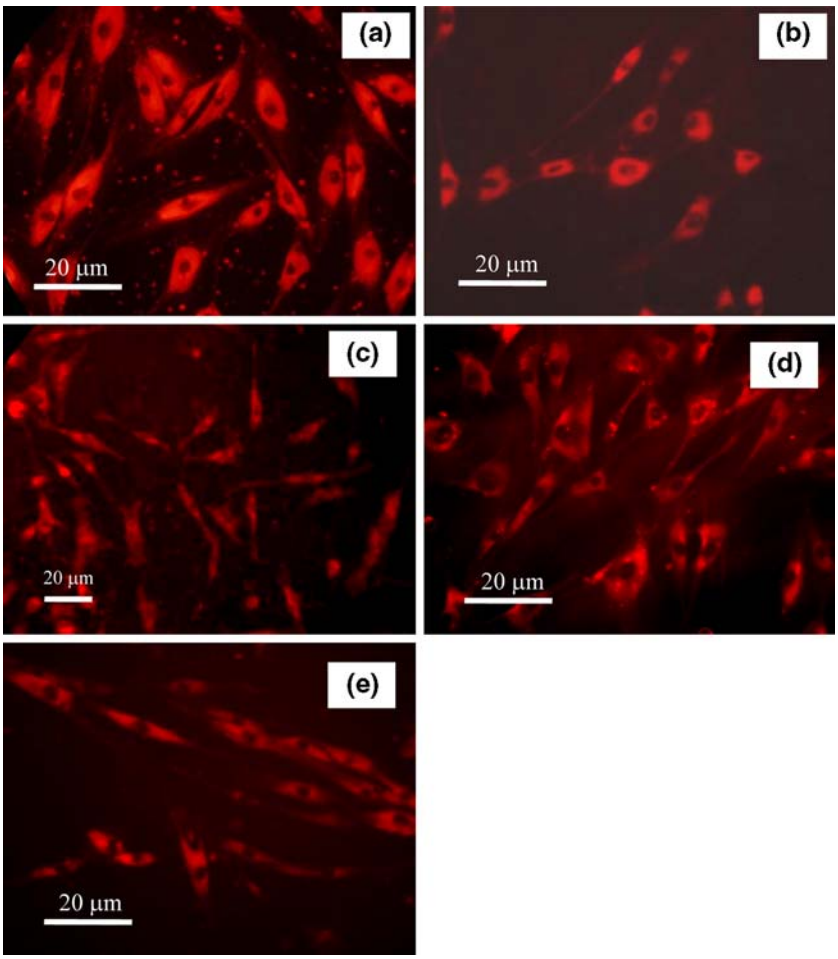

Fig. 4 Morphology of fibroblast cells adhering on different surfaces after 3 days of culture: (**a**) reference sample, (**b**) Ti, (**c**) Ti-34, (**d**) Ti-7, (**e**) Ti-col

The highest viability is associated with the smallest corrosion rate observed for the surface treated by MOCVD under atmospheric pressure (Ti-34). The corresponding oxide layer is ~1 μm thick and is slightly anatase (56%) than rutile (44%) richer. Sul [8] has shown that bone tissue reactions were strongly reinforced with oxidized titanium implants, the oxide being of the anatase type and thicker than 600 nm, with a porous surface structure. In vitro as soon as the implantation is completed, the cells tend to migrate inside the pores of the passive film. This interpenetration certainly facilitates the adhesion between implant and tissue [30]. Another study [33] has demonstrated that crystalline titanium oxides had a slightly higher activation of the clotting cascade but lower platelet adhesion than nanocrystalline and amorphous titanium oxides. Buhler et al. [34] showed that a fine nanotopography probably induces the cells to produce more focal adhesion points. In the present study, cell viability increases in the following order: Ti < Ti-col < Ti-7 < Ti-34 (2).

Cell spreading as determined by Sigma Scan software increases in the following order: Ti ≤ Ti-34 < Ti-col < Ti-7 (3). Despite a spreading value for Ti-34 close to that of Ti, all in vitro data as abstracted by relations (1), (2) and (3) show that the MOCVD treatment increases the biocompatibility.

The two MOCVD-treated samples used for cell growth experiments lead to somewhat different results both for cell spreading and cell viability. These two samples differ in allotropic composition (Table 1). They have rather different nanostructures as dramatically shown on Fig. 1. The films also differ in roughness (Table 1) even if the substrate roughness is about the same (ca. 1 μm) for either sample. From these two examples alone, it is difficult to

Table 4 Cell viability and cell spreading versus titanium surface modification

	Control	Ti	Ti-col	Ti-7	Ti-34
Viability	100	74.3	70.0	75.7	78.9
Spreading	13.9	8.5	11.7	23.6	8.8

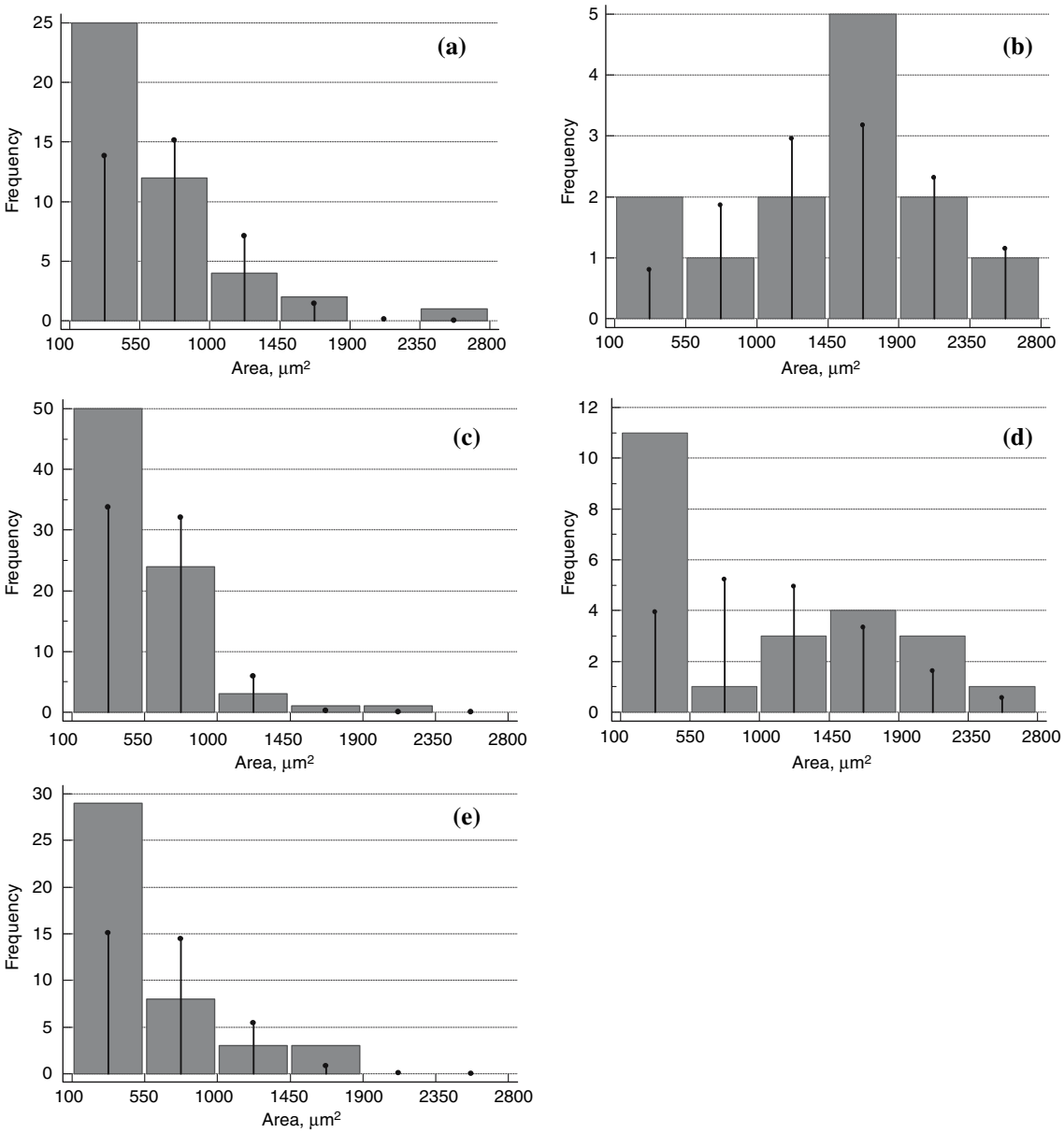

Fig. 5 Histograms for the area of the fibroblasts: (**a**) reference surface, (**b**) Ti, (**c**) Ti-34, (**d**) Ti-7, (**e**) Ti-col

make correlations between structural parameters (at the nano- and the micro-scale) and biological behaviour. Moreover, respective effects of the different techniques of sterilization have been the subject of several discussions in the last decade [6, 32, 35–37] and should also be considered.

Conclusion

1. Two surface treatments have been applied to pure titanium samples: (i) coating by collagen from a collagen gel, (ii) coating by TiO_2 grown by MOCVD. The MOCVD process has been applied under different experimental conditions thus leading to quite different coating morphologies both on the nano- and the micro-scale. The corrosion rate is very small for every sample, indicating very good film stability in the following order: Ti < Ti-col < Ti-10 < Ti-30.
2. In comparison with untreated titanium surface, both the bio-inert coating and the bio-interactive one lead to enhanced biological behaviour in terms of cell spreading and cell viability as estimated with in vitro cultured human dermal fibroblasts. Cell growth data correlated with electrochemical data reveal a better viability for the treated Ti samples. The highest

viability is associated with the smallest corrosion rate observed for the surface that has been treated by MOCVD under atmospheric pressure (Ti-34).

3. Cell spreading as determined by Sigma Scan software increases in the following order: Ti ≤ Ti-34 < Ti-col < Ti-7. The results of this study suggest that the nanostructural features may influence biocompatibilty and deserve further thorough studies.

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